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Blood pressure lowering effect of selected pyrimidine derivatives mediated through inhibition of calcium channel: A computational approach

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Abstract: Pyrimidine 2, 4, 6-trione derivatives are known to have L-type calcium channel blockade activity due to which they are quite effective in cardiovascular diseases along with cancer, epilepsy and inflammatory disorders. The chemoinformatics prediction for test compounds: 5-(3-Hydroxybenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-5), 5-(4-Hydroxybenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-8), 5-(3-Chlorobenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-9) and 5-(4-Chlorobenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-10) was investigated. The druglikeliness and pharmacokinetic properties (PKs) of test compounds calculated using Molinspiration & Swiss ADME online servers. These test drugs subjected to molecular docking analysis and molecular dynamic (MD) simulation to calculate their binding energies with hypertensive and platelet aggregatory proteinaceous targets and their stability against calcium channel. The druggability and PKs of selected compounds exhibited that these compounds could be represented as potential candidates for further development into antihypertensive-like agents. The docking results indicated that binding energies ranged between -5 and -8.8 kcal/mol. Compounds showed good binding energies against calcium channels (CC) and subjected to molecular dynamic simulation to assess the stability of protein-ligand complex. The results showed that all the ligands form stable complexes with the CC, though SR-9 and SR-10 had enhanced stability when compared to SR-5 and SR-8.

Keywords: Pyrimidines, computational pharmacology, docking, simulation.

INTRODUCTION

Cardiovascular diseases (CVDs) have endured one of the primary reasons of death globally. They account for about 1/3 of total deaths, totaling ~17 million yearly worldwide (Akter *et al*., 2022). Hypertension, the persistent raise of blood pressure (BP) above 140/90 mmHg (systolic/diastolic BP, respectively), is considered one of the critical risk factors for the genesis of CVDs (Mills *et al* 2020). Hypertension is frequently termed a 'silent killer,' distressing 1 billion individuals worldwide and triggering up to 9 million deceases every year (WHO, 2013). It is the most common and main contributor to the development of CVDs, comprising coronary heart disease, ischemia and hemorrhagic stroke (Franklin and Wong, 2013). CVDs mostly affect the inhabitants of low- and middle-income countries (Alwan, 2011). It is expected that over 80% of CVDs deaths occur in developing countries, with a numeral that may reach 23.3 million in the next 15 years (Mathers and Loncar, 2006: Anand *et al*., 2020).

In spite of numerous therapeutic interventions to control or treat hypertension, no single agent completely solves the problem. Mostly, patients must take several drugs, each of which targets several mediators predisposed for high BP. Such agents might comprise angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, calcium channel blockers (CCBs), beta-blockers, alpha-blockers, alpha 2 agonists, non-selective alpha and beta blockers and vasodilators to manage CVDs and to relieve symptoms (Zhuang *et al*., 2016). There is a dire need to find newer therapeutic agents. Nitrogen-containing compounds like pyrimidines are privileged heterocyclic scaffolds due to their biological and pharmacological activities (Sabatini *et al*., 2012: Patil, 2018). Pyrimidine being a 6-membered cyclic compound comprising 4-carbon and 2-nitrogen atoms and is pharmacologically inactive, but its synthetic derivatives have an important role in modern medicine. Pyrimidine derivatives have anti-microbial, analgesic, antiviral, anti-inflammatory, anti-tumor, anti-malarial, diuretic, hypnotic, hepatoprotective and anti-obesity activities (Ma *et al*., 2012: Kang *et al*., 2013: Singh and Chouhan, 2014).

Computer-aided drug design (CADD) or virtual screening in combination with wet lab procedures play vital role in the discovery of new drug moities (Sabe *et al*., 2021). CADD is mainly valuable exclusively in three major domains: Identification of utmost appropriate compounds from enormous libraries of probably lead compounds, addition of a suitable groups in the lead compounds for

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creating it more appropriate for new drug discovery and by the addition of pharmacophore properties, designing the new entity from a target structure. In light of existing project the interactions of test compounds against the several targets of CVDs was evaluated through docking and *in silico* absorption, distribution metabolism and excretion (ADME) parameters (Khalid *et al*., 2018). Docking, *in silico* procedure, is employed for the assessment of the binding affinity among two substance like protein-protein and ligands-macromolecules (Colombo *et al*., 2007). Molecular dynamic (MD) simulations is a powerful tool and are extensively used to study the immovability of macromolecules or their complexes and to improve the quality of speculative models (Raghav *et al*., 2012a, 2012b). Procedures like molecular encounters that have been usually considered by Brownian dynamics simulations have also been addressed by MD simulation in the former decade (Lao *et al*., 2019).

The present study purposes to examine the blood pressure lowering potential of selected compounds (fig. 1): mediated through inhibition of calcium channel (CC) by employing computational techniques.

MATERIALS AND METHODS

Druglikeliness

Molinspiration server v2022.04 and SwissADME (version 14.9.29, 2013, www.chemaxon.com) employed to foresee and compare numeral of rotatable bonds, hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), molecular weight (MW), lipid water partition coefficient (logP) and topological polar surface area (TPSA) through both servers. These considerations aid in appraisal of drug likeliness in accordance with Lipinski's rule of five (Lipinski *et al*., 2012). According to this rule, for any compound to be a good drug candidate, it must have a) MW≤500 Da, increasing weight lessens the test compounds concentration at the surface of intestinal lining, which ultimately decreases absorption. b) HBD≤5, c) HBA≤10. The increasing number of HB can decrease separation from the aqueous into the lipid bilayer membrane for penetration through simple diffusion. d) logP value≤5, high logP value results in poor absorption. TPSA being another determinant of fraction absorption would be≤140 which relates with passive transport through membranes and total rotatable bonds fewer than 10 (Jin *et al*., 2018).

Calculation of Pharmacokinetic properties (PKs)

PKs such as absorption, distribution, metabolism and excretion (ADME) are considered dire in development of drug because they govern the possessions of an operative oral drug. The structural properties of four substances were drawn on the SwissADME server using the structure drawing tool and the online software Molinspiration. Some significant ADME descriptors were considered like expected aqueous solubility, blood brain barrier (BBB) penetration, cytochrome p-450 2D6 (CYP2D6), human intestinal absorption (HIA) (Daina *et al*., 2017).

Molecular docking

Molecular docking accomplished by Auto Dock tool and PyRx (Duhovny *et al*., 2012). Docking executed and estimated on the bases of energy (E) values (Kcal/mol). Lowest E- value (Kcal/mol) with best poses selected for valuation through Biovia Discovery Studio Visualizer (DSV). Each complex evaluated in 2D form to evaluate maximum binding interactions formed among test compounds and amino acid residues. Target proteins selected were endothelin receptor type B (ER), ATP-sensitive potassium channel (K channel), Calcium channel (CC), guanylyl cyclase (GC), beta 1-receptor (β1), beta 2-receptor (β2), angiotensin-converting enzyme (ACE), angiotensin receptor (AT_1), renin, mineralocorticoid receptor (MCR), COX-1, glycoprotein-IIb/IIIa receptor (GP-IIb/IIIa), glycoprotein-VI receptor (GP-VI), purino receptor (P_2Y_{12}), phosphodiesterase-5 (PDE-5), prostacyclin receptor (PGI_2) with PDB-ID's; 5XPR, 5WUA, 4DEY, 3UVJ, 2YCW, 3SN6, 4CA5, 4ZUD, 3VYD, 3VHU, 3N8X, 2VDM, 2GI7, 4PXZ, 2H42 and 4F8K respectively were utilized for docking analysis with test compounds. These macromolecules were rescued from Research Collaboratory for Structural Bioinformatics (RCSB) in protein data bank (PDB) format. Standards drugs Bosentan, Bethanidine, Nifedipine, Isosorbide dinitrate, Propranalol, Carvedilol, Captopril, Losartan, Aliskiren, Spironolactone, Aspirin, Tirofiban, Hinokitiol, Clopidogrel, Dipyridamole, Beraprost acquired from https://pubchem.ncbi.nlm.nih. DSV used for post-docking analysis and schematic illustration of HB and amino acid residues participated in bonding of best pose.

Molecular dynamics (MD) simulation

MD simulations executed for 100 nanoseconds using Desmond, a Package of Schrödinger LLC (Vijayakumar *et al*., 2018). The preliminary phase of protein-ligand complexes for MD simulation were attained after docking. These studies offer a likelihood of binding status in static environments whereas simulations performed in physiological milieu. The macromolecule-ligand interactions were processed through Protein Preparation Wizard, which furthermore involved optimization and minimization of interactions. All systems were organized through the System Builder tool. Solvent model was designated as transferable intermolecular interaction potential 3 points. The OPLS-2005 force field was employed in the simulation (Shivakumar *et al*., 2010: Soumia *et al*., 2022). The models tend to neutralized via addition of counter ions. To mimic the physiological environment, 0.15M salt (sodium chloride) was used. The NPT ensemble (Isothermal-Isobaric) with 300 K

temperature and 1 atm pressure was selected for whole simulation. The trajectories were saved after every 100ps during analysis and strength was assessed by computing the root mean square deviation (RMSD) of the macromolecules and ligand. The Desmond simulations trajectories were analyzed. RMSD, root mean square, fluctuation (RMSF) and protein-ligand contacts were calculated from MD trajectory analysis.

RESULTS

In-silico analysis

Drug like Properties and PKs

SR-5, SR-8, SR-9, SR-10 and nifedipine exhibits logP, TPSA, MW, HBA and HBD values of 0.24, 95.5, 232, 4, 3, 0.25, 95.5, 232, 4, 3, 1.21, 75.27, 250, 3 ,2, 1.2, 75.27, 250, 3, 2 and 3.07, 110.46, 346, 8, 1 respectively through Molinspiration and Swiss ADME (table 1). PKs represented as fig. 2-5.

Molecular docking

Selected test compounds showed -5 to -8.8 Kcal/mol affinity against ER, K-channel, CC, GC, β_1-receptor**,** β_2-receptor, ACE, AT_1, Renin, MCR, COX-1, GP-IIb/IIIa, GP-VI, P_2Y_{12}, PDE 5 and PGI2. Drug-protein complex of best pose with E-values, number of hydrogen bonds and residues are presented in table 2. 2D interactions of test compounds and reference drugs with selected targets are shown in figs. S1-S16.

DISCUSSION

In-silico approaches are fast and cost-effective procedure, required computer assisted techniques and software. Druglikeliness and PKs of treatments is a noteworthy factor in selection of drug and to measure its effectiveness as a therapeutically advantageous agent. Therefore, in current project the physico-chemical parameters were evaluated to measure the PK properties. Lipinski's rule of 5 stated that the drug must have MW of≤500 Da, HBD≤5, HBA≤10 and oil-water partition coefficient log P≤5. If drug fulfil this criteria then it's considered as an orally effective drug. The drug substances which fails to comply more than one of above stated directions may experiences difficulty with bioavailability. In present study none of the test drug is violating Lipinski rule of 5 when evaluated through Molinspiration and Swiss ADME server, as already reported through Molinspiration earlier (Irshad *et al*., 2021a) here we have compared through these servers. Therefore, *in silico* computational investigation has been capable to find some promising compounds predicted to have good bioavailability which could ascertain to be valuable in the managing of hypertension. Further PKs were considered to recognize the real ADME delinquent behind the test substance in order to avoid later failure of the test substance. Therefore, bearing all ADME factors together, selected compounds fulfilled the ADME descriptor measures at the optimal level.

Docking prototypes have simulated as a worthwhile substitute to wet lab research techniques particularly at preliminary stages where chemical compounds are several but assets are limited (Daina *et al*., 2017). Docking is an imperative method. It is basically not a impartial technique and works best if treated as a supplementary procedure alongside with other *in silico* methods as well as *in vitro* and *in vivo* experimentations (Goodsell *et al*., 1996). Though there is a definite need for new pharmacological research, *in silico* exploration is an operational and encouraging tool for ascertaining pharmacological utility of both new and existing drugs (Merk *et al*., 2018). It is a primary tool used to measure affinity of ligands to particular protein targets involved in hypertension and platelet aggregation. Intermolecular interactions play a significant role in stabilizing ligand(s) energetically in macromolecules pocket. These interactions (Morris and Wilby, 2008) may be in the form of HB, Van der Waal forces and electrostatic interactions. HB interactions are essential for internal stabilization, recognition and molecular movement. Lower binding energy discloses decreased energy of desolvation that represents immovability of ligand protein complex (Deganutti *et al*., 2017). E-values and bonding (hydrogen) play vital part in calculation of binding affinity of ligand and macromolecules. On the basis of E-values, order of SR-5 affinity against selected targets: PDE 5> Ca Channel >ER> ACE> Renin> COX1 > AT_I> MCR> β_2- receptor> GC> P_2Y_{12}> β_1-receptor> GP-IIb/IIIa> K-channel> PGI_2> GP-IV. Order of SR-8: ER> Ca Channel> MCR> PDE 5> AT_I> Renin> COX 1> ACE> β_2- receptor> GC> β_1-receptor> P_2Y_{12}> K-Channel> GP-IIb/IIIa> GP-IV> PGI_2. Order of SR-9: ER> PDE 5> Ca Channel> ACE> Renin> MCR> AT_I> K-Channel> β_1-receptor> GC> COX 1> GP-IIb/IIIa> β_2- receptor> PGI_2> P_2Y_{12}> GP-IV. Order of SR-10: ER> PDE 5> Ca Channel> ACE> AT_I> Renin> COX 1> GC> PGI_2> MCR> GP-IIb/IIIa> K-Channel> P_2Y_{12}> β_2- receptor> GP-IV> β_1-receptor. Virtual study is accompanied presenting results of ligand compared with standard drugs, obtained from RCSB and PubChem data base. The primary emphasis of the current study is to indicate significance of docking exploration and ascertain compounds encompassing pyrimidine nucleus for the management of hypertension possibly through calcium channel blockade activity. By using *in silico* CADD methodologies, the activity of selected compounds against CVDs targets was also assessed. Docking was validated.

MD simulations were accomplished for 100 ns using Desmond. The simulation trajectories were analyzed. RMSD, RMSF and protein-ligand interactions were anticipated from MD trajectory analysis. The RMSD plot of the complexes CC+SR-5 and CC+SR-8 indicate that the complexes do reach stability throughout the simulation and the RMSD plot of the complexes CC+SR-9 and CC+SR-10 indicate that the proteins reached steadiness at 5 ns.

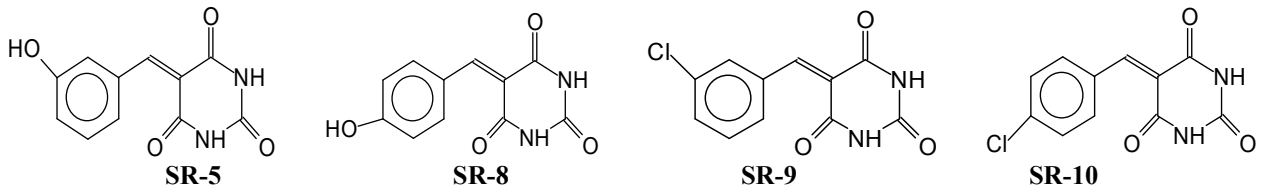

Fig. 1: Chemical structures of selected compounds: 5-(3-Hydroxybenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-5), 5-(4-Hydroxybenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-8), 5-(3-Chlorobenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-9) and 5-(4-Chlorobenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-10).

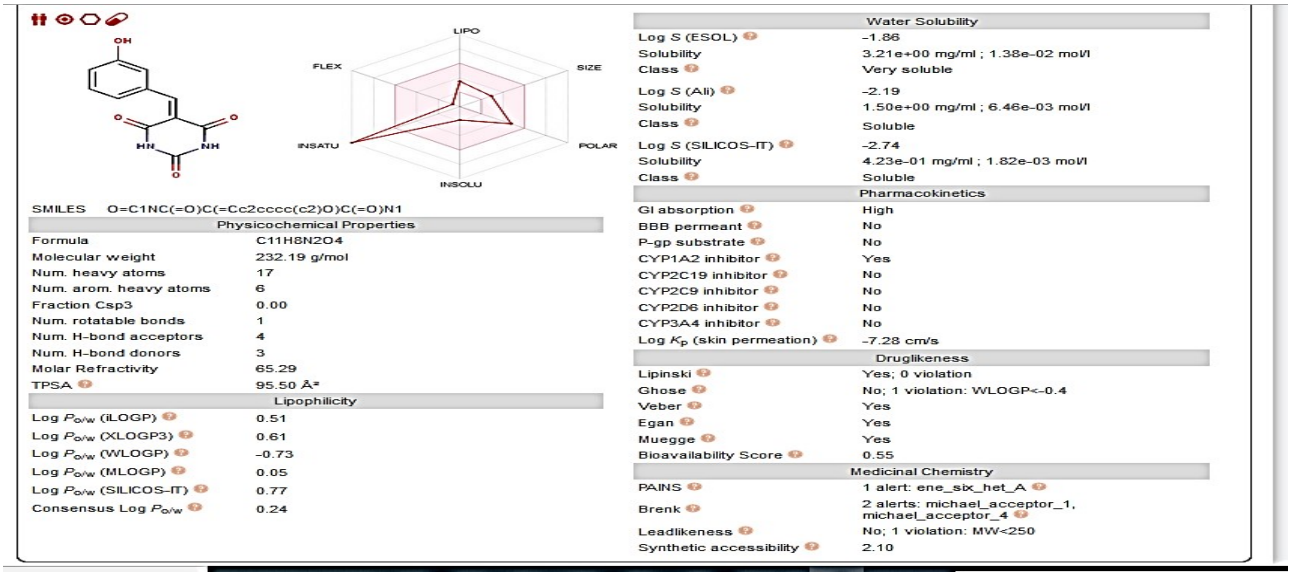

Fig. 2: The assessment of physicochemical parameters and pharmacokinetics properties (PKs) of the 5-(3-Hydroxybenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-5) using Swiss target prediction software. The various properties includes bioavailability, absorption, distribution and water solubility. SR-5 followed the Lipinski, Veber, Egan, Muegge rule while showed the bioavailability score of 0.55.

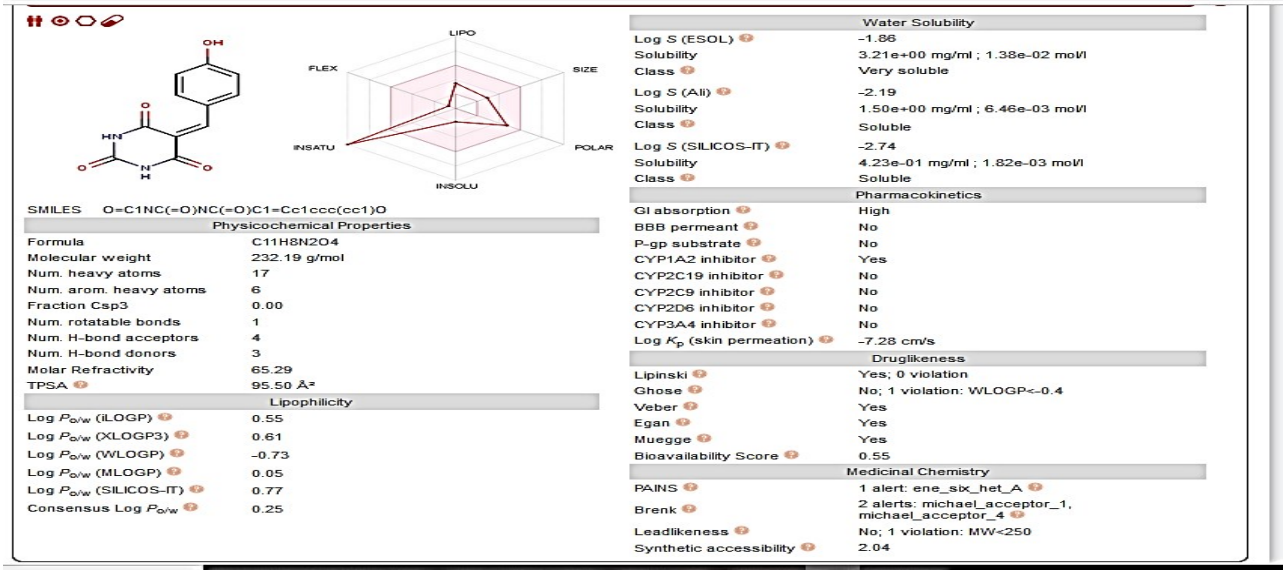

Fig. 3: The assessment of physicochemical parameters and pharmacokinetics properties (PKs) of the 5-(4-Hydroxybenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-8) using Swiss target prediction software. The various properties includes bioavailability, absorption, distribution and water solubility. SR-5 followed the Lipinski, Veber, Egan, Muegge rule while showed the bioavailability score of 0.55.

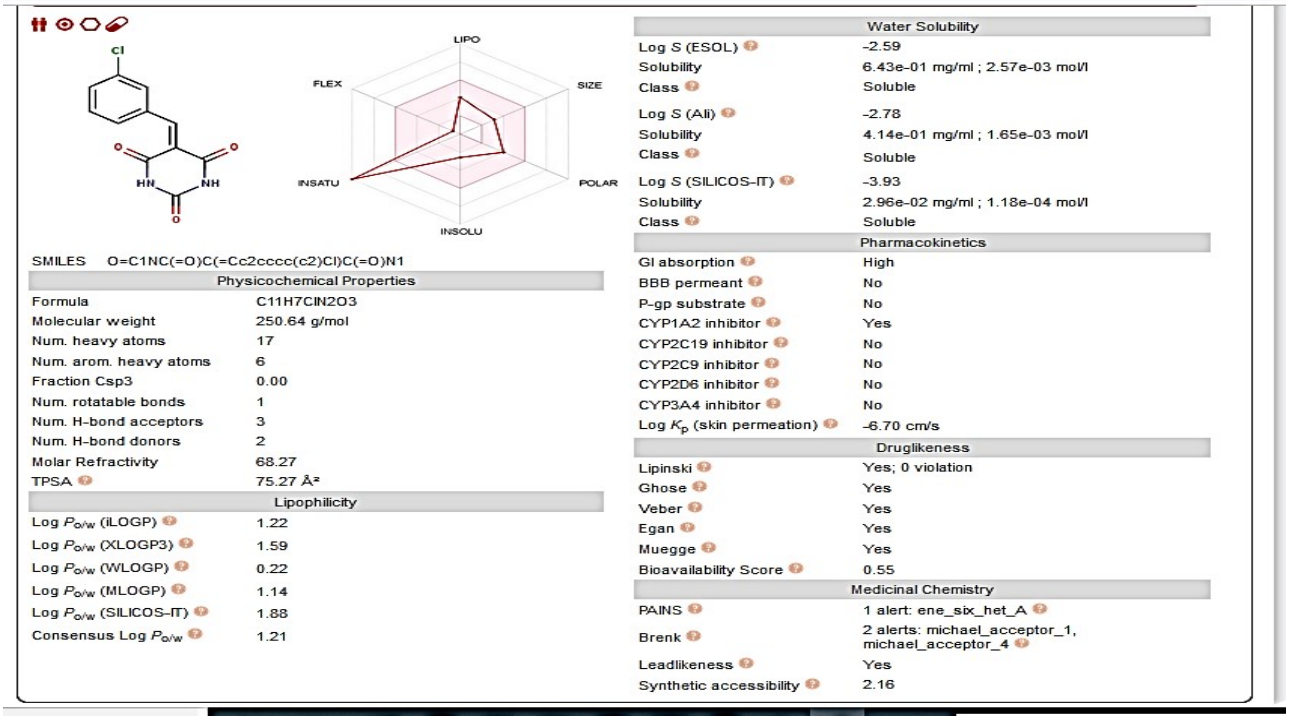

Fig. 4: The assessment of physicochemical parameters and pharmacokinetics properties (PKs) of the 5-(3-Chlorobenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-9) using Swiss target prediction software. The various properties includes bioavailability, absorption, distribution and water solubility. SR-5 followed the Lipinski, Veber, Egan, Muegge rule while showed the bioavailability score of 0.55.

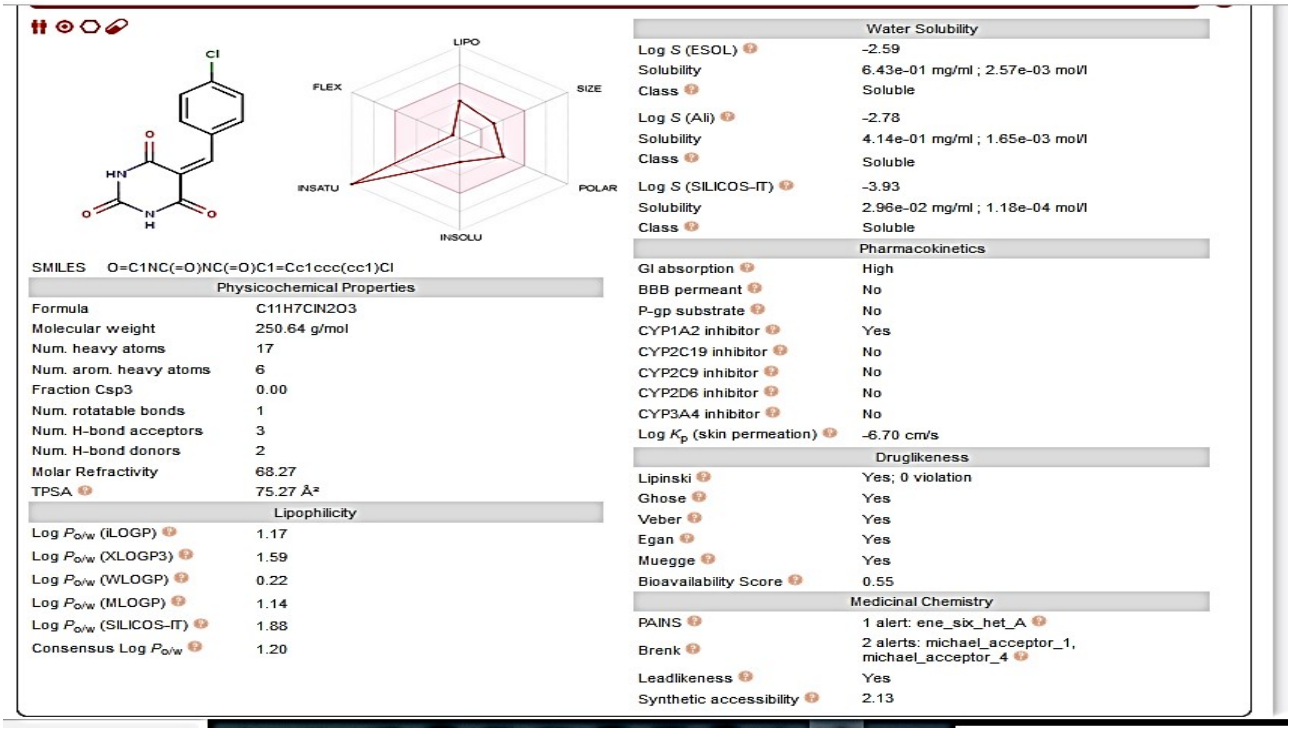

Fig. 5: The assessment of physicochemical parameters and pharmacokinetics properties (PKs) of the 5-(4-Chlorobenzylidene)-2, 4, 6(1H, 3H, 5H)-pyrimidinetrione (SR-10) using Swiss target prediction software. The various properties includes bioavailability, absorption, distribution and water solubility. SR-5 followed the Lipinski, Veber, Egan, Muegge rule while showed the bioavailability score of 0.55.

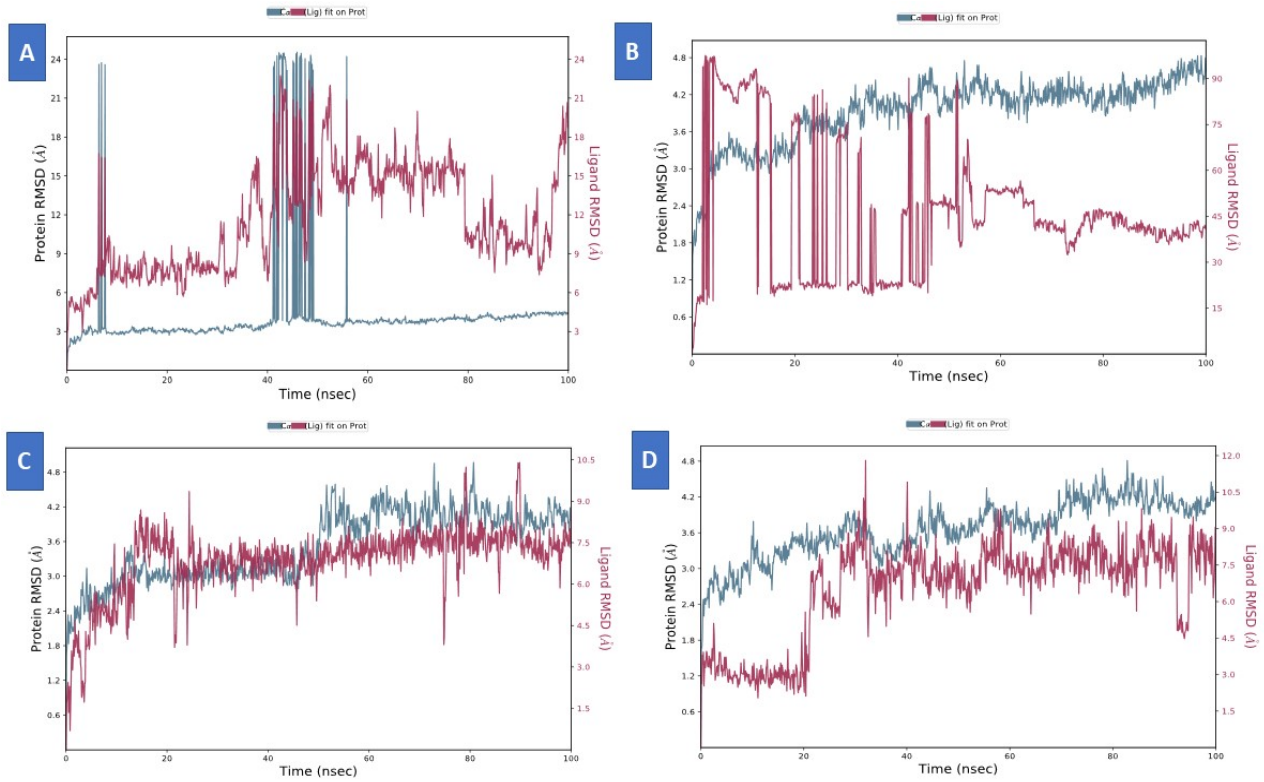

Fig. 6: Root mean square deviation (RMSD) of the C-alpha atoms of protein (calcium channel) and the ligands (A: SR-5, B: SR-8, C: SR-9 and D: SR-10) with time. The left Y-axis shows the variation of protein RMSD through time. The right Y-axis shows the variation of ligand RMSD through time.

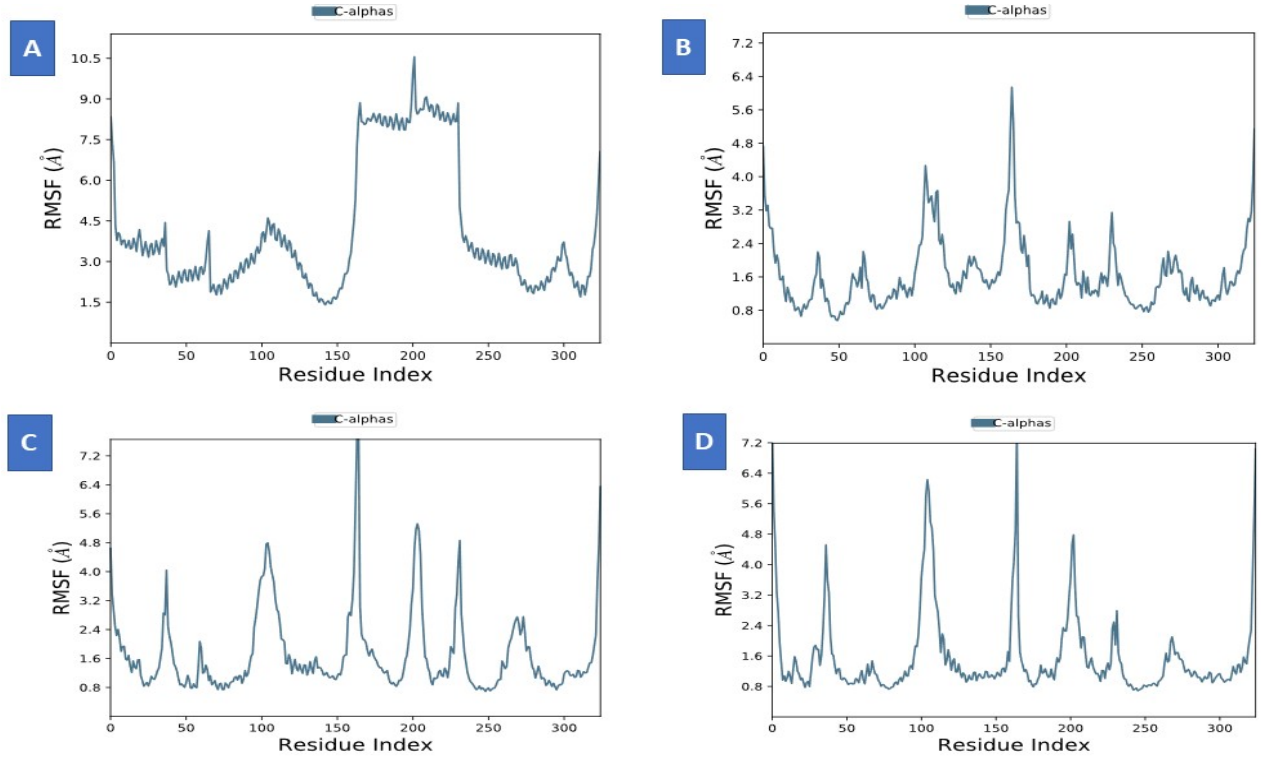

Fig. 7: Residue wise Root Mean Square Fluctuation (RMSF) of protein (calcium channel) complexed with ligands (A: SR-5, B: SR-8, C: SR-9 and D: SR-10).

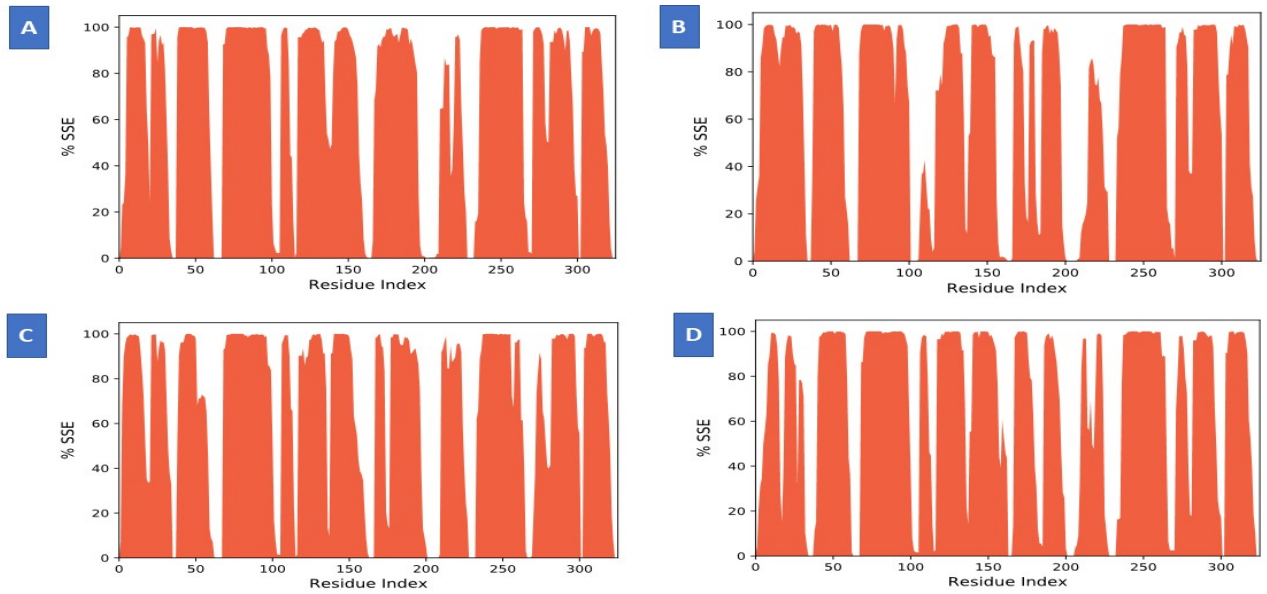

Fig. 8: Protein Secondary Structure Element (SSE) distribution by residue index throughout the protein structures complexed with SR-5 (A), SR-8 (B), SR-9 (C) and SR-10 (D). Red columns indicate alpha helices and blue columns indicate beta-strands.

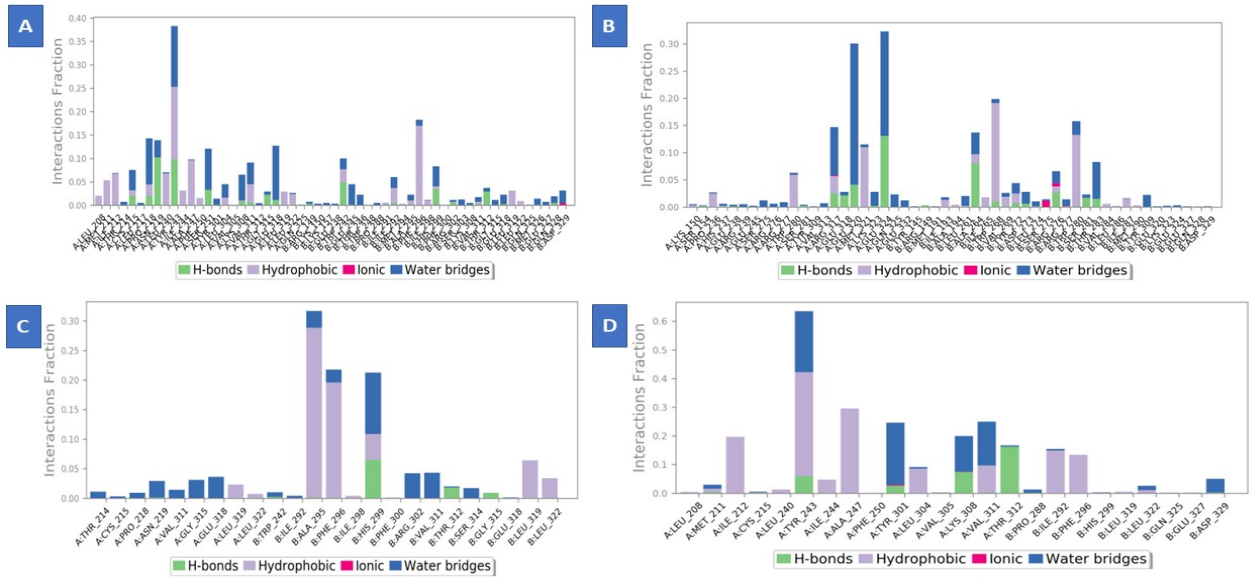

Fig. 9: Protein-ligand contact histogram (protein structures complexed with SR-5 (A), SR-8 (B), SR-9 (C) and SR-10 (D).

Table 1: Drug-like properties of 5-(3-Hydroxybenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-5), 5-(4-Hydroxybenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-8), 5-(3-Chlorobenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-9) and 5-(4-Chlorobenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-10) and nifedipine using Molinspiration and SwissADME models.

Ligands	LogP	TPSA	MW	HBA	HBD	No. of violations	No. of rotatable bonds
SR-5	0.24	95.5	232	4	3	0	1
SR-8	0.25	95.5	232	4	3	0	1
SR-9	1.21	75.27	250	3	2	0	1
SR-10	1.2	75.27	250	3	2	0	1
Nifedipine	3.07	110.46	346	8	1	0	6

LogP= Lipid water partition coefficient, TPSA= Topological polar surface area,
MW= molecular weight, HBA= hydrogen bond acceptor, HBD=hydrogen bond donor.

Table 2: Best pose dock analysis showing E-values (Kcal/mole) and hydrogen bond formed by interaction of 5-(3-Hydroxybenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-5), 5-(4-Hydroxybenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-8), 5-(3-Chlorobenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-9), 5-(4-Chlorobenzylidene)-2, 4, 6(*1H, 3H, 5H*)-pyrimidinetrione (SR-10) and standard drugs against targets: endothelin receptor type B (ER), potassium channel (K-channel), calcium channel (CC), guanylyl cyclase enzyme (GC), beta 1 (β_1)- receptor, beta 2 (β_2)- receptor, angiotensin converting enzyme (ACE), angiotensin receptor (AT_1), renin, mineralocorticoid receptor (MCR), cyclo-oxygenase 1 (COX_1), glycoprotein-IIb/IIIa (GP-IIb/IIIa) receptor, glycoprotein-VI (GP-VI) receptor, purino receptor (P2Y12), phosphodiesterase 5 (PDE 5) and prostacyclin (PGI_2).

Target Proteins	SR-5			SR-8			SR-9			SR-10			Standard Drugs			
	E-Value (kcal /mol)	H-bond	Bonding residues	E-Value (kcal /mol)	H-bond	Bonding residues	E-Value (kcal /mol)	H-bond	Bonding residues	E-Value (kcal /mol)	H-bond	Bonding residues	Drugs Name	E-Value (kcal /mol)	H-bond	Bonding residues
ER	-8.3	2	TYR 475, HIS 461	-8.8	2	PRO 334, TRP 356	-8.9	2	SER102	-9.4	2	PRO 334, TRP 356	Bosentan	-6	1	ILE 101
K-channel	-6.3	0	0	-6.2	1	THR 33	-7.7	2	THR 107, ILE 100	-6.5	1	THR 33	Bethanidine	-6	2	GLU 70 GLU 254
CC	-8.6	2	TYR 156, SER 102	-8.8	2	GLY 18 ASP 14	-8.5	2	GLY 18, ASP 14	-8.7	3	TYR 66 TYR 52 ILE 33	Nifedipine	-7	1	PHE 329
GC	-7	5	LEU 91 LEU 94 SER 96 GLN 181 PRO 179	-6.9	-2	GLU 90 LEU 91	-7	3	PRO 179 LEU 91 LEU 94	-7.3	3	TYR 66 ILE 33 TYR 52	Isosorbide dinitrate	-8.8	0	0
β1-receptor	-6.8	2	GLU 70 LYS 47	-6.9	2	GLU 70 GLU 254	-7.3	1	GLU 70	-5.9	0	0	Propranolol	-6.9	4	THR40 THR 134 GLN 129 GLN 133
β2-receptor	-7.1	4	THR 40 THR 134 GLN 133 GLU 129	-7.3	4	GLU 129 GLN 133 THR 134 HIS 41	-6.8	2	GLU 129 GLN 133	-6.3	4	HIS 41 THR 134 GLN 133 GLU 129	Carvedilol	-7	4	GLU 129 GLN 133 THR 134 HIS 41
ACE	-7.9	2	LYS 896 THR 943	-7.4	4	GLY 1024 ASN 1025 LYS 896 LYS938	-8	1	LYS 896	-8	2	THR 943 LYS 896	Captopril	-9	1	THR 288
AT1	-7.6	2	TRP84, THR 88	-7.6	2	TYR 87, TYR 35	-7.8	2	GLU 129 GLN 133	-7.7	4	HIS 41 THR 134 GLN 133 GLU 129	Losarten	-8	1	ILE 101
Renin	-7.8	3	THR 85, THR 18, SER 230	-7.6	2	PRO 118, THR 85	-8	3	THR 85, THR 18, SER 230	-7.7	2	THR 85, PRO 118	aliskiren	-7.2	1	GLU 70
MCR	-7.5	2	THR 880,SER 936	-8.4	0	0	-8	2	ALA 773, ARG 817	-7.2	2	TYR 804, THR 880	Spironolactone	-6	3	MET 106 LYS 112 LYS 52
COX_1	-7.8	2	ASP 228 PHE 275	-7.5	3	TYR 204 TRP 214	-7	2	TRP 214 PHE 250	-7.5	2	TRP 214 PHE 250	Aspirin	-6.9	2	ASP 228 PHE 275
GP-IIb/IIIa	-6.8	2	ARG 46 ILE 47	-6	2	ARG 46 ILE 47	-7	2	ARG 46 ILE 47	-7.2	2	ARG 46 ILE 47	Tirofiban	-6	1	THR 288
GP-VI	-5.6	5	SER 87 SER 258 LYS 257 PHE 331 TYR 324	-6	3	PRO 82 LEU 254 TYR 324	-5	3	ARG 334 TYR 324 PHE 331	-6	2	PHE 331 ARG 334	Hinokitiol	-7	3	TYR 334 GLU 282 THR 283
P2Y12	-7	2	SER 288 TYR 32	-6.5	6	LYS 131 THR 132 LYS 125 ARG 128 ARG 122 VAL 234	-6	3	ARG 122 THR 132 PRO 129	-6.5	1	TYR 105	Clopidogrel	-7	4	LYS 532 GLN 372 SER 121 TRP 214 TYR 204
PDE 5	-8.6	4	TYR 612, LEU 765, GLN 775,HIS 613	-8.3	3	ASP 764, TYR 612, ILE 768	-8.7	2	ASP 764, TYR 612	-8.7	2	PRO 334 TRP356	Dipyridamole	-7	2	GLU 70 LYS 471
PGI2	-6.3	5	LEU 91 LEU 94 SER 96 GLN 181 PRO 179	-6	2	GLU 90 LEU 91	-6.1	3	PRO 179 LEU 91 LEU 94	-7.3	3	TYR 66 ILE 33 TYR 52	Beraprost	-7.9	3	SER 251 TYR 204 TRP 214

Amino acids are: alanine (ALA), arginine (ARG), asparagine (ASN), aspartic acid (ASP), cysteine (CYS), glutamine (GLN), glutamic acid (GLU), glycine (GLY), histidine (HIS), isoleucine (ILE), lysine (LYS), methionine (MET), phenylalanine (PHE), proline (PRO), serine (SER), threonine (THR), tryptophan (TRP), tyrosine (TYR) and valine (VAL).

After that, changes in RMSD values endure inside 1.5 Å during the simulation period, which is quite satisfactory. Ligand fit to protein RMSD values varies within 1.5 Å and 2.0 Å after being stable for CC+SR-9 and CC+SR-10 respectively. These showed that ligand remains firmly bound to active site of target during simulation. CC+SR-9 and CC+SR-10 are more stable, which is also confirmed earlier in *In-Vivo* models that these compounds have good activity against CC as compared to SR-5 and SR-8 (Irshad *et al*., 2021b). Fig. 2 demonstrate the residue wise RMSF value of the protein binding to selected test compounds. The residues presenting greater peaks resemble to loop areas, as recognized from MD trajectories (fig. 3), or N and C-terminal regions. Low RMSF values represents the stability of ligand with the protein. Most important interactions are HB and hydrophobic interactions, as illustrated in fig. 4. A:ANS_219, A:TYR_243 are the most significant ones in the forms of H-bonds for CC+SR-5, A:GLU_324 and A:ALA_264 are the essential ones in light of H-bonds for CC+SR-8, B:ALA_295, B:PHE_296 and B:HIS_299 are the most imperative ones in the form of hydrophobic interactions and H-bonds for CC+SR-9 and A:TYR_243, A:ALA_274 and A:THR_312 are essential in terms of hydrophobic interactions and H-bonds CC+SR-10. The bar charts were stabilized during the course of the trajectory: that is, a value of 1.0 recommends that for 100% of the simulation time, the particular interaction was preserved. Values above 1.0 are promising as few protein residue can make numerous interactions of the same subtype with the ligand.

CONCLUSION

Selected compounds fulfill the criteria of druglikeliness evaluated through molinspiration and SwissADME server. PKs parameters of selected compounds fulfilled the ADME descriptor criteria at the optimum level including solubility, bioavaialability, BBB penetration, PGP substrate etc. Docking results showed that compounds exhibit binding energies between -5 to -8.8 kcal/mol against targets accounts in hypertension and platelet aggregation. MD simulation represents that the agents remains firmly bound to target during the simulation and SR-9 and SR-10 are more stable.

REFERENCES

Akter H, Rashid MM, Islam MS, Hossen MA, Rahman A, Algheshairy RM, Almujaydil MS, Alharbi HF and Alnajeebi AM (2022). Biometabolites of *Tamarindus indica* play a remarkable cardioprotective role as a functional food in doxorubicin-induced cardiotoxicity models. *J. Funct. Foods,* **96**(2): 1756-4646.

Alwan A (2011). Global status report on non-communicable diseases: World Health Organization 2010: **176**

Anand S, Bradshaw C and Prabhakaran D (2020). Prevention and management of CVD in LMICs: Why do ethnicity, culture and context matter?. *BMC Med.,* **18**(1): 7.

Colombo G, Soto P and Gazit E (2007). Peptide self-assembly at the nanoscale: A challenging target for computational and experimental biotechnology. *Trends Biotechnol.,* **25**(5): 211-218.

Daina A, Michielin O and Zoete V (2017). Swiss ADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*., **7**: 42717

Deganutti G, Zhukov A, Deflorian F, Federico S, Spalluto G, Cooke RM, Moro S, Mason JS and Bortolato A (2017). Impact of protein-ligand solvation and desolvation on transition state thermodynamic properties of adenosine A_{2A} ligand binding kinetics. *In silico Pharmacol.,* **5**(1): 16.

Duhovny DS, Rossi A, Avila-Sakar A, Kim SJ, Muriel JV, Strop P, Liang H, Krukenberg KA, Liao M, Kim HM, Sobhanifar S, Dotsch V, Rajpal A, Pons J, Agard DA, Cheng Y, Sali A (2012). A method for integrative structure determination of protein-protein complexes. *Bioinformatics,* **28**(24)*:* 3282-3289.

Franklin SS and Wong ND (2013). Hypertension and cardiovascular disease: Contributions of the Framingham heart study. *Global Heart*, **8**(1): 49-57.

Goodsell David S, Morris Garrett M and Olson Arthur J (1996). Automated docking of fexible ligands: Applications of AutoDock. *J. Mol. Recognit*., **9**(1): 1-5.

Irshad N, Khan A-u, Alamgeer, Khan S and Iqbal MS (2021b). Antihypertensive potential of selected pyrimidine derivatives: Explanation of underlying mechanistic pathways. *Biomed. Pharmacother.,* **139**: 111567.

Irshad N, Khan A-u, Shah FA, Nadeem H, Ashraf Z, Tipu MK and Li S (2021a). Antihyperlipidemic effect of selected pyrimidine derivatives mediated through multiple pathways. *Fundam Clin Pharmacol*., **35**(6): 1119-1132.

Kang S, Cooper G, Dunne SF (2013). Structure-activity relationship of N, N-disubstituted pyrimidinetriones as $Ca_{V1.3}$ calcium channel-selective antagonists for Parkinson's disease. *J. Med. Chem.,* **56**(11): 4786-4797.

Khalid S, Zahid MA, Ali H, Kim YS and Khan S (2018). Biaryl scafold-focused virtual screening for anti-aggregatory and neuroprotective effects in Alzheimer's disease. *BMC Neurosci.,* **19**(1):74

Lao Z, Chen Y, Tang Y and Wei G (2019). Molecular dynamics simulations reveal the inhibitory mechanism of dopamine against human islet amyloid polypeptide (hiapp) aggregation and its destabilization effect on hiapp protofibrils. *ACS Chem. Neurosci.,* **10**(9): 4151-4159.

Lipinski CA, Lombardo F, Dominy BW and Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.,* **46**(1-3): 3-26.

Ma L, Xie C and Ran Y (2012). Synthesis and biological evaluation of 5-benzylidenepyrimidine 2, 4, 6(1H, 3H, 5H) -trione derivatives for the treatment of obesity related nonalcoholic fatty liver disease. *J. Med. Chem.,* **55**(22): 9958-9972.

Mathers CD and Loncar D (2006). Projections of global mortality and burden of disease from 2002 to 2030, *PLoS Medicine,* **3**(11): 442.

Merk D, Grisoni F, Friedrich L, Gelzinyte E and Schneider G (2018). Scafold hopping from synthetic RXR modulators by virtual screening and de novo design. *Med. Chem. Comm.,* **9**(8): 1289-1292.

Mills KT, Stefanescu A and He J (2020). The global epidemiology of hypertension. *Nat. Rev. Nephrol.,* **16**(4): 223-237.

Morris GM and Wilby LM (2008). Molecular docking. *Methods Mol Biol.,* **443**: 365-382

Patil SB (2018). Biological and medicinal significance of pyrimidines: A review. *IJPSR,* **9**(1): 44-52.

Raghav PK, Verma YK and Gangenahalli GU (2012a). Molecular dynamics simulations of the Bcl-2 protein to predict the structure of its unordered flexible loop domain. *J. Mol. Model.,* **18**(5): 1885-1906.

Raghav PK, Verma YK and Gangenahalli GU (2012b). Peptide screening to knockdown Bcl-2's anti-apoptotic activity: Implications in cancer treatment. *Inter. J Biol. Macro.,* **50**(3): 796-814.

Sabatini S, Gosetto F and Serritella S (2012). Pyrazolo [4, 3-c] [1, 2] benzothiazines 5, 5-dioxide: A promising new class of *Staphylococcus aureus* NorA efflux pump inhibitors. *J. Med. Chem.,* **55**(7): 3568-3572.

Sabe VT, Ntombela T, Jhamba LA, Maguire GEM, Govender T, Naicker T and Kruger HG (2021). Current trends in computer aided drug design and a highlight of drugs discovered via computational techniques: A review. *Eur. J. Med. Chem.,* **224**: 113705.

Shivakumar D, Williams J, Wu Y, Damm W, Shelley J and Sherman W (2010). Prediction of absolute solvation free energies using molecular dynamics free energy perturbation and the OPLS force field. *J. Chem. Theory Comput*., **6**(5): 1509-1519.

Singh R and Chouhan A (2014). An Overview of biological importance of pyrimidine. *W. J. Pharm. Pharma. Sci*., **3**: 574-597.

Soumia M, Hajji H, El-Mzibri M, Younes FZ, Mohammed B and Benaissa M (2022). *In silico* molecular modeling studies to identify novel potential inhibitors of HPV E6 Protein. *Vaccines,* **10**(9): 1452.

Vijayakumar S, Manogar P, Prabhu S and Singh RAS (2018). Novel ligand-based docking; Molecular dynamic simulations; and absorption, distribution, metabolism and excretion approach to analyzing potential acetylcholinesterase inhibitors for Alzheimer's disease. *J. Pharma. Ana.*, **8**(6): 413-420.

WHO (2013). A global brief on hypertension, silent killer, global public health crisis; Geneva, Switzerland, WHO/DCO/WHD/2013.2

Zhuang X, Liao L and Dong X (2016). Design, synthesis and antihypertensive activity of curcumin-inspired compounds via ace inhibition and vasodilation, along with a bioavailability study for possible benefit in cardiovascular diseases. *Drug Des. Devel. Ther*., **10**: 29-139.